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Attorney Docket No. 55325-8148.US06

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Date: September 29, 2003

Lynnea B. Anderson
Lynnea B. Anderson

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

MARTIN, et al.

APPLICATION NO.: 10/016,324

FILED: December 10, 2001

FOR: **THERAPEUTIC LIPOSOME COMPOSITION AND METHOD OF PREPARATION**

EXAMINER: KISHORE, G

ART UNIT: 1615

CONFIRMATION NO: 4133

Appeal Brief Transmittal

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Sir:

Further to the Notice of Appeal submitted July 28, 2003, enclosed herewith are the following:

☒ Applicant's Appeal Brief in triplicate.

☐ Petition for -Month Extension of Time.

☒ Fee (37 C.F.R. § 1.17(c)): ☐ Small Entity: $160.00
☒ Large Entity: $320.00

- ☒ Enclosed is a check for $320.00 covering the above fee.
- ☐ Please charge the above fee(s) to Deposit Account No. 50-2207. This paper is provided in triplicate.
- ☒ Please charge any underpayment for timely consideration of this paper to Deposit Account No. 50-2207.

☒ Applicant petitions for an Extension of Time if necessary for timely filing of this Brief.

Respectfully submitted,

Jacqueline F. Mahoney

Date: September 29, 2003

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16/Appeal Brief (3)
Attorney Docket No. 55325-8148.US06
PATENT
9/m 9-29-03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:
Martin and Zalipsky
APPLICATION NO.: 10/016,324
FILED: December 10, 2001
FOR: **THERAPEUTIC LIPOSOME COMPOSITION AND METHOD OF PREPARATION**

EXAMINER: Kishore
ART UNIT: 1615
CONFIRMATION NO: 4133

Bet 10-14-03

APPELLANT'S BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Mail Stop Appeal Brief - Patents

Sir:

This is an appeal to the Board of Appeals and Interferences from the decision of Examiner Kishore mailed March 27, 2003 in which pending claims 29-59 stand in final rejection.

The present paper is Appellants' Appeal Brief submitted in compliance with 37 C.F.R. §1.192.

REAL PARTY IN INTEREST

The real party in interest is Alza Corporation, a subsidiary of Johnson & Johnson.

RELATED APPEALS AND INTERFERENCES

Appellants are not aware of other appeals or interferences which would directly affect or be directly affected by or have a bearing on the Board's decision in the present appeal.

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STATUS OF THE CLAIMS

The application was initially filed with 28 claims. Claims 1-28 were cancelled and claims 29-59 were added by Preliminary Amendment filed December 10, 2001. The final rejection of pending claims 29-59, as presented in Appendix A, is appealed.

STATUS OF AMENDMENTS

Appellants' response dated July 28, 2003 and submitted after final rejection was entered and made of record.

SUMMARY OF INVENTION

The present invention relates to a method of administering a therapeutic agent via inhalation (page 32, line 21). The therapeutic agent is entrapped (page 4, lines 20-21) in liposomes formed of vesicle-forming lipids (page 9, line 4). The liposomes further have a coating of hydrophilic polymer chains on the liposome outer surface (page 9, lines 9-10).

ISSUES

The issues on appeal are:

1. Whether claims 29-31, 33-37, 39, and 40-45 are anticipated under 35 U.S.C. §102(e) by Marshall *et al.* (U.S. Patent No. 5,939,401; hereinafter "Marshall *et al.*").

2. Whether claims 29-30, 34-37, 39-41, 44-49, and 55 are obvious under 35 U.S.C. §103(a) over Mihalko *et al.* (PCT Publication No. WO 86/06959 to Liposome Technology, hereinafter "Mihalko *et al.*") in combination with Klibanov *et al.* (J. Liposome Research, 2(3):321-334, 1992, hereinafter "Klibanov *et al.*").

3. Whether claims 29-31, 33-37, 39, and 40-45 are patentable under 35 U.S.C. §103(a) over Marshall *et al.* by itself or in combination with Mihalko *et al.*

4. Whether claims 31-33 are patentable under 35 U.S.C. §103(a) as over Marshall *et al.* by itself or in combination with Mihalko *et al.*, further in view of Gao and Huang (*BBRC*, 179(1):280-285, 1991, hereinafter "Gao and Huang").

5. Whether claims 49-57 are patentable under 35 U.S.C. §103 over Mihalko *et al.* in combination with Klibanov *et al.*, further in view of Chestnut *et al.* (U.S. Patent No. 5,800,815, hereinafter Chestnut *et al.*), DeFrees *et al.* (U.S. Patent No. 5,604,207, hereinafter DeFrees *et al.*) and Applicants' statements of prior art.

GROUPING OF CLAIMS

With regard to all issues in this Appeal, claims 29-59 stand or fall together. Claims 29-59 are presented in Appendix A.

ARGUMENTS

Claims 29-59 stand rejected over various combinations of six documents. A summary of the cited documents is provided in Appendix B.

1.0 Regarding Novelty rejection over Marshall *et al.*

The standard for lack of novelty, that is, for anticipation, is one of strict identity. To anticipate a claim for a patent, a single prior source must contain all its essential elements. M.P.E.P. § 2131

The present invention includes administering, via inhalation, liposomes formed of vesicle-forming lipids and having a coating of hydrophilic polymer chains on the liposome outer surface, where said liposomes have an entrapped therapeutic agent.

The teaching of Marshall *et al.* fails to show at least three of the following presently claimed elements: (1) a liposome; (2) a liposome having a coating of hydrophilic polymer chains; and (3) a liposome having an entrapped therapeutic agent.

With respect to the first element, it is clear from the teaching in Marshall *et al.* that discrete liposomes are not formed with the cationic amphiphiles. The cationic amphiphiles

form "complexes" rather than liposomes, as evidenced by the following description in the Marshall *et al.* document:

(i) On Col. 15, lines 19-26, Marshall *et al.* describe preparing a dispersion of a cationic amphiphile; contacting the dispersion with a biologically active molecule to form a complex between said amphiphile and said molecule.

(ii) On Col. 33, lines 33-49, Marshall *et al.* state that while cationic amphiphiles can form liposomes, "the cationic amphiphiles of the invention need not form highly organized vesicles in order to be effective, and in fact can assume (with the biologically active molecules to which they bind) a wide variety of loosely organized structures."

(iii) On Col. 33, lines 62-65, Marshall *et al.* state "owing to the potential for leakage of contents therefrom, vesicles or other structures formed from numerous of the cationic amphiphiles are not preferred by those skilled in the art in order to deliver low molecular weight biologically active materials."

These comments are consistent with the thermodynamic expectation that mixing a negatively charged biologically active molecule with a cationic amphiphile will initially result in charge-charge interaction between the two species and a loose, nonordered complex of the two species will form. Upon neutralization of the charge, and in the presence of any excess cationic amphiphiles, bilayer formation around the entire complex can occur. However, the mere presence of a bilayer does not imply liposome formation, since a liposome is considered to those of skill in the art to be a discrete, defined particle.

This view is supported by the disclosure of Marshall *et al.* where PEG-DMPE is added to "stabilize" the cationic amphiphile-molecule complex, "preventing further aggregation of formed amphiphile/DNA complexes" (Col. 53, lines 46-50). Were liposomes formed by the cationic amphiphile complex, aggregation would not be a problem since liposomes are stable, discrete particles with little tendency to aggregate.

Further, the disclosure by Marshall *et al.* that vesicles or other structures (e.g., other than complexes) formed from numerous of the cationic amphiphiles are not preferred because of leakage of the entrapped contents (Col. 33, lines 62-66) is also

consistent with Appellants' assertion that liposomes are not formed from the cationic amphiphiles. Liposome formation requires that the lipids have the ability to pack into spherical particles and leakage of entrapped small molecular weight contents indicates a lack of ability to pack appropriately for true liposome formation.

Marshall *et al.* further fail to teach a liposome having a coating of hydrophilic polymer chains on the liposome outer surface. Foremost, as shown above, Marshall *et al.* fail to teach a liposome in general and thus cannot be said to teach a liposome with a coating of hydrophilic polymer chains. However, even if the cationic amphiphiles were said to form liposomes, Marshall *et al.* do not teach use of a lipid-PEG conjugate for liposome formation. In Marshall *et al.*, the sole disclosure of lipid-PEG conjugate is for stabilization of a complex of a DNA-cationic amphiphile. Marshall *et al.* teach the use of an amphiphile-containing film that may include PEG-DMPE as a stabilizing ingredient (Col. 53, lines 36-42). One of skill in the art would in no way take this as a teaching of a liposome with a hydrophilic polymer coating. Thus, there is no disclosure of a liposome having a coating of polymer chains.

Marshall *et al.* finally fail to teach a liposome having an entrapped therapeutic agent. For the reasons given above, Marshall *et al.* fail to teach a liposome in general and thus cannot be said to teach a liposome with an entrapped agent. The biologically active molecule of Marshall *et al.* is described as polynucleotides, such as genomic DNA, cDNA and mRNA, ribosomal RNA, antisense polynucleotides, and ribozymes (Col. 17, lines 20-24). It is imminently clear from the disclosure of Marshall *et al.*, and well supported in the literature, that polynucleotides cannot be "entrapped" in liposomes due to their large size. Moreover, when cationic lipids are used for liposome formation, the charge interaction of the lipids with the polynucleotides inhibits liposome formation and entrapment of the agent. These molecules are complexed with an amphiphile (Col. 15, lines 19-26), but are not entrapped due to the large size of the molecule-liposome complexes, charge interactions, and rather poor *in vivo* transfection efficiencies. While Marshall *et al.* teach that the amphiphiles may form liposomes (Col. 33, lines 33-34), Marshall *et al.* in no way teach discrete liposomes with an entrapped therapeutic agent, as in the present invention (Col.

33, lines 43-47). In fact, Marshall *et al.* state that the liposomes formed of the amphiphiles "are not preferred" due to the fact that liposomes formed from the amphiphiles may leak the biologically active molecule (Col. 33, lines 62-66).

Conclusion: All the Essential Elements are not Taught

Because the cited Marshall *et al.* reference does not teach all of the essential elements of the present invention, Appellants urge the Board to overturn the rejection of claims 29-31, 33-37, 39, and 40-45 based on this reference.

2.0 Regarding the Combination of Mihalko *et al.* and Klibanov *et al.*

2.1 Is there Motivation to Combine Mihalko *et al.* and Klibanov *et al.*?

"Obviousness can only be established by combining or modifying the references of the prior art to produce the claimed invention when there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." MPEP §2143.01

Mihalko *et al.* describe a method and system for inhalation administration of a drug in a suspension of liposomes (Abstract). The liposomes of Mihalko *et al.* are administered by inhalation to the lung, where the drug is released from the liposomes into the respiratory tract (page 25, lines 9-13 and 20-22). The drug, sans liposome, is subsequently taken up systemically from the site of deposition in the pulmonary region of the respiratory tract (page 10, line 31 through page 11, line 2).

Klibanov *et al.* teach coating liposome surfaces with a hydrophilic layer to create long-circulating liposomes for systemic delivery.

To arrive at the claimed invention, one would need to modify the liposomes of Mihalko *et al.* to include a coating of hydrophilic polymer chains. There is simply no motivation in either of the cited references to effect this modification of Mihalko *et al.*, for the following reasons.

First, it is clear from the teaching of Klibanov *et al.* that the purpose of providing a coating of hydrophilic polymer chains on a liposome is to extend the blood circulation lifetime of the liposomes. That is, the hydrophilic polymer shields the liposomes from recognition and uptake by the reticuloendothelial system (RES) (page 324, lines 19-22). However, the liposomes of Mihalko *et al.* are administered not into the blood circulation where RES uptake is a problem but are administered by inhalation to the lung (page 4, lines 16-19 and 24-27). Mihalko *et al.*, based on the teaching of Klibanov *et al.*, would find no reason to modify the liposomes to have an extended blood circulation lifetime since blood circulation life is of no concern where the liposomes are only delivered to the lung.

Nor is motivation for the modification found in the teaching of Klibanov *et al.* Klibanov *et al.* is concerned solely with intravenous administration and nowhere mentions other forms of administration, much less inhalation, or that liposomes with a coating of hydrophilic polymers chains would be useful, suitable, or desirable for inhalation administration. As noted above, the liposomes of Mihalko *et al.* are administered by inhalation to the lung. The drug is released from the liposomes <u>into the pulmonary region</u> of the respiratory tract by efflux from the liposome (page 25, lines 9-13) and <u>only the drug</u> enters the blood for circulation (page 25, lines 17-22). As the purpose of the liposome with a hydrophilic polymer coating is to protect the liposome from the RES for longer circulation, one would not be motivated to modify the liposomes of Mihalko *et al.* as the liposomes <u>do not</u> circulate.

Because there is no motivation, either explicit or implicit, to combine the teachings of Mihalko *et al.* and Klibanov *et al.*, Appellants urge the Board to overturn the rejection of claims 29-30, 34-37, 39-41, 44-49, and 55 based on a combination of these documents.

2.2 Is there an Expectation of Success?

Another criterion to establish a prima facie case of obviousness is that "there must be a reasonable expectation of success." M.P.E.P. § 2143. In the present case, neither of the references, alone or in combination provide a reasonable expectation of success that liposomes having a coating of hydrophilic polymer chains would be effective for administration by inhalation, for the following reason.

One important requirement for successful delivery of liposomes to the lung is particle size. As noted by Mihalko *et al.* "[r]educing liposome particle size may be important in achieving efficient aerosolization of the liposomes" (page 13, lines 24-26). A particle with a larger spherical volume is more difficult to administer to the lung itself and tends to be retained in the upper airway Coating a liposome with hydrophilic polymer chains increases the spherical volume of the particle[1], counter to the expressly stated requirement of Mihalko *et al.* for efficient administration to the lung.. Thus, in this respect, it would undesirable to coat the liposomes of Mihalko *et al.* with a polymer, since such a coating a liposome can drastically change the size of the liposome particle and impact delivery to the lung.

Further, according to Mihalko *et al.* "[r]educing liposome particle size may be important in achieving efficient aerosolization of the liposomes" (page 13, lines 24-26). Since coating liposomes with hydrophilic polymer chains increases the liposomal particle size, liposomes having a coat of hydrophilic polymer chains may not aerosolize as desired. There is simply no way of knowing from these references whether liposomes having a hydrophilic polymer coating would be able to be administered by inhalation, much less be effective.

Because there is no expectation of success that the combined teachings of Mihalko *et al.* and Klibanov *et al.*, Appellants urge the Board to overturn the rejection of

[1]Hristova and Needham, in STEALTH LIPOSOMES, Lasic and Martin, Eds., CH 5: "Physical Properties of Polymer-Grafted Bilayers", CRC Press, 1995. Copy provided with Appellants response filed January 10, 2003.

claims 29-30, 34-37, 39-41, 44-49, and 55 based on a combination of these documents.

3.0 Regarding Marshall *et al.* by itself or in combination with Mihalko *et al.*

Marshall *et al.* are concerned with intracellular delivery and teach a particular type of lipid, a cationic amphiphile, as a vehicle to achieve intracellular delivery of a compound, particularly a nucleic acid (Col. 1, lines 31-33). The cationic amphiphiles when mixed with a nucleic acid form a complex, as opposed to a liposome (Col. 3, lines 17-20). Marshall *et al.* disclose that low molecular weight compounds leak from the structure formed by the cationic amphiphiles, suggesting that the cationic amphiphiles are incapable of packing to achieve liposome formation (Col. 33, lines 62-66). Moreover, Marshall *et al.* nowhere show or suggest a liposome having a coating of hydrophilic polymer chains on the liposome outer surface. At most, Marshall *et al.* teach a nucleic acid-cationic amphiphile complex stabilized with a PEG derivatized lipid.

As discussed above in 2.1, Mihalko *et al.* are silent regarding hydrophilic polymer chains and utterly fail to show or suggest coating a liposome with hydrophilic polymer chains. Further, as noted above, Mihalko *et al.* emphasize the importance of a small liposomes size for aerosolization and delivery to the lung. Addition of polymer chains to the outer surface increases the spherical volume of the liposome. Thus, it would not be obvious based on the teaching in Mihalko *et al.* to add hydrophilic polymer chains to the liposome.

Nor would it have been obvious to administer the complexes of Marshall *et al.* by inhalation as taught by Mihalko *et al.* The various modes of administration for delivering therapeutic agents (*e.g.,* intravenous, subcutaneous, topical, etc). are not readily interchangable since each mode has specific difficulties and requirements. In fact, Mihalko *et al.* recite some of the difficulties with other forms of administration in that some drugs "are susceptible to breakdown in the gastrointestinal tract, or which otherwise cannot be administered orally" (page 1, lines 32-34) showing that modes of administration must be chosen carefully based on the limitations of the mode and the

therapeutic agent. It is also well known that administration of some drugs by inhalation is simply not feasible. In fact, Mihalko *et al.* state that "[a] related problem is the limitation on the amount of drug that can be administered safely at each inhalation, particularly in the case of a drug which has unwanted systemic side effects" (page 3, lines 5-9).

Thus, the teachings of Marshall *et al.* and Mihalko *et al.* fail to show or suggest a method of delivering via inhalation liposomes having a surface coating of hydrophilic polymer chains and an entrapped compound as presently claimed. Accordingly, Appellants urge withdrawal of the rejections based on Marshall *et al.* alone or in view of Mihalko *et al.*

4.0 Regarding the Combination of Marshall *et al.* by itself or in combination with Mihalko *et al.* further in view of Gao and Huang

This combination of documents is used to reject dependent claims 31-33. Claims 31-33 are dependent on claim 29, which defines over the combination of Marshall *et al.* and Mihalko *et al.* for the reasons above.

The addition of Gao and Huang does not change the analysis given above as Gao and Huang make no mention of hydrophilic polymer chains or of coating a liposome with hydrophilic chains. Gao and Huang are cited merely for the inclusion of dimethylaminoethane carbamoyl cholesterol in a liposome formulation.

Accordingly, Appellants urge withdrawal of the rejections based on Marshall *et al.* by itself or in combination with Mihalko *et al.* further in view of Gao and Huang.

5.0 Regarding the Combination of Mihalko *et al.* in view of Klibanov *et al.* and further in view of Chestnut *et al.*, DeFrees *et al.* and Applicants' statements of prior art

As noted above, any attempt to combine the teachings of Mihalko *et al.* and Klibanov *et al.* fails for lack of motivation to combine and/or a reasonable expectation of success. More specifically, nothing in the teachings of Mihalko *et al.* or Klibanov *et al.* would motivate one skilled in the art to modify the liposomes of Mihalko *et al.*, that are

designed for administration to the lung, with the teaching of the Klibanov *et al.* to provide a coating of hydrophilic polymer chains since such chains serve to extend blood circulation lifetime of intravenously administered liposomes and this is not of concern for liposomes delivered by inhalation.

Moreover, Mihalko *et al.* state the importance of small liposome size for inhalation delivery, and to modify liposomes with polymer chains increases the overall spherical volume, expressly in contrast to the teaching in Mihalko *et al.*

The teachings of Chestnut *et al.*, DeFrees *et al.*, and Applicant's statements do not make up for these problems in attempting to combine the teachings of Mihalko *et al.* with Klibanov *et al.* Chestnut *et al.* and DeFrees *et al.* are concerned with liposomes having a targeting ligand on the surface of the liposome. Neither reference makes any reference to a coating of hydrophilic polymer chains on the liposome outer surface or of administration by inhalation. Applicants' statement that targeting ligands are knows also does not make up for the problems in combination of Mihalko *et al.* with Klibanov *et al.*

Accordingly Appellants request the Board to overturn the rejection of claims 49-57.

CONCLUSIONS

In view of the foregoing remarks, Appellants submit that the pending claims are in condition for allowance and patentably define over the prior art, and urge the Board to overturn the Examiner's rejections.

Respectfully submitted,

Jacqueline F. Mahoney

Date: Sept. 29, 2003

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APPENDIX A: CLAIMS ON APPEAL

29. A method of administering a therapeutic agent, comprising,

administering via inhalation liposomes formed of vesicle-forming lipids and having a coating of hydrophilic polymer chains on the liposome outer surface, said liposomes having an entrapped therapeutic agent.

30. The method of claim 29, wherein the vesicle-forming lipid is selected from the group consisting of hydrogenated soy phosphatidylcholine, distearoylphosphatidylcholine, sphingomyelin, diacyl glycerol, phosphatidyl ethanolamine, phosphatidylglycerol, distearyl phosphatidylcholine, and distearyl phosphatidylethanolamine.

31. The method of claim 29, wherein said liposomes further contain a cationic lipid shielded by said coating of hydrophilic polymer chains, said cationic lipid being effective to impart a positive liposome-surface charge.

32. The method of claim 31, wherein the cationic lipid is selected from the group consisting of 1,2-dioleyloxy-3-(trimethylamino) propane, N-[1-(2,3,-ditetradecyloxy)propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide, N-[1-(2,3,-dioleyloxy)propyl]-N,N-dimethyl-N-hydroxy ethylammonium bromide, N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride; 3β[N-(N',N'-dimethylaminoethane) carbamoly] cholesterol; and dimethyldioctadecylammonium.

33. The method of claim 31, wherein the cationic lipid is a neutral lipid derivatized with a cationic lipid.

34. The method of claim 29, wherein said hydrophilic polymer coating is composed of hydrophilic polymers selected from the group consisting of polyvinylpyrrolidone,

polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, polyethyleneglycol, and polyaspartamide.

35. The method of claim 34, wherein said hydrophilic polymer coating is composed of polyethylene glycol chains having a molecular weight of between about 500 Daltons and about 10,000 Daltons.

36. The method of claim 29 wherein between about 1 mole percent and about 20 mole percent of the vesicle-forming lipids are derivatized with said hydrophilic polymer chains.

37. The method of claim 29, wherein at least a portion of the hydrophilic polymer chains are joined by a chemically releasable bond.

38. The method of claim 37, wherein said releasable bond is a disulfide bond.

39. The method of claim 37, wherein said releasable bond is a pH sensitive chemical linkage.

40. The method of claim 29, wherein the liposomes are composed of between about 70-90 mole percent hydrogenated soy phosphatidylcholine, about 1-20 mole percent distearylphosphatidylcholine derivatized with polyethyleneglycol and about 1-50 mole percent cholesterol.

41. The method of claim 29, wherein the liposome is about 0.1 to about 10 microns.

42. The method of claim 29, wherein the agent entrapped in the lipid vesicles is a polynucleotide capable of expressing a selected protein, when taken up by a target cell.

43. The method of claim 29, wherein the agent entrapped in the liposomes is an oligonucleotide or oligonucleotide analog effective for sequence-specific binding to cellular RNA or DNA.

44. The method of claim 29, wherein the agent entrapped in the liposomes is selected from the group consisting of DNA, proteins, and peptides.

45. The method of claim 29, wherein the agent entrapped in the liposomes is selected from the group consisting of antibiotics, antivirals, and antitumor drugs.

46. The method of claim 29, wherein said liposomes further contain a ligand attached to the distal end of at least a portion of said hydrophilic polymer chains.

47. The method of claim 29, wherein the liposomes further include a ligand attached the polar head group of at least a portion of the vesicle-forming lipids of the liposome.

48. The method of claim 46 or 47, wherein the ligand is an antibody or an antibody fragment.

49. The method of claim 48, wherein the ligand is a Fab' fragment of an antibody.

50. The method of claim 48, wherein the ligand is a single chain Fv antibody.

51. The method of claim 46 or 47, wherein the ligand specifically binds to an extracellular domain of a growth factor receptor.

52. The method of claim 51, wherein the receptor is selected from the group consisting of epidermal growth factor receptor, basic fibroblast growth factor receptor and vascular endothelial growth factor receptor.

53. The method of claim 46 or 47, wherein the ligand binds a receptor selected from the group consisting of E-selectin receptor, L-selectin receptor, P-selectin receptor, folate receptor, CD4 receptor, αβ integrin receptors and chemokine receptors.

54. The method of claim 46 or 47, wherein the ligand is selected from the group consisting of folic acid, pyridoxal phosphate, sialyl Lewis^x, transferrin, epidermal growth factor, basic fibroblast growth factor, vascular endothelial growth factor, VCAM-1, ICAM-1, PECAM-1, and RGD peptides.

55. The method of claim 46 or 47, wherein the ligand is selected from the group consisting of water soluble vitamins, apolipoproteins, insulin, galactose, Mac-1, PECAM-1/CD31, fibronectin, osteopontin, RGD sequences of matrix proteins, HIV GP 120/41 domain peptomers, GP120 C4 domain peptomers, T cell tropic isolates, SDF-1 chemokines, Macrophage tropic isolates, anti-cell surface receptor antibodies or fragments thereof, pyridoxyl ligands, RGD peptide mimetics, and anti-E-selectin Fab.

56. The method of claim 55, wherein the anti-cell surface receptor antibodies or fragments thereof is selected from the group consisting of anti-selectin and anti-VEGF pyridoxyl.

57. The method of claim 55, wherein the pyridoxyl ligand is selected from the group consisting of pyridoxal, pyridoxine, pyridoxamine, pyridoxal 5'-phosphate and N-(4'-pyridoxyl)amines.

58. The method of claim 29, wherein said liposomes are further comprised of a lipid derivatized by a diblock copolymer composed of a hydrophobic polymer chain covalently bound to the lipid and a hydrophilic polymer chain, the hydrophobic and hydrophilic chains being joined by a bond effective to release the hydrophilic polymer chains in response to an existing or an induced physiologic condition, thereby exposing the hydrophobic polymer chains.

59. The method of claim 58, wherein said hydrophobic polymer is selected from the group consisting of polypropylene oxide, polyethylene, polypropylene, polycarbonate, polystyrene, polysulfone, polyphenylene oxide and polytetramethylene ether.

APPENDIX B

Summary of the Cited Documents

MARSHALL ET AL. relate to cationic amphiphiles complexed with therapeutic molecules for intracellular delivery. The biologically active molecules described are polynucleotides, such as genomic DNA, cDNA and mRNA, ribosomal RNA, antisense polynucleotides, and ribozymes (Col. 17, lines 20-24). A dispersion of the amphiphile is prepared and contacted with a biologically active molecule to form a complex between the amphiphile and the molecule. Cells are then contacted with the complex to facilitate transfer of the molecules into the cells (Col. 15, lines 19-26). The complexes may be administered "by onsite delivery using additional micelles, gels and liposomes" (Col. 34, lines 27-31) One type of structure that may be formed by amphiphiles is the liposome, however, "owing to the potential for leakage of contents therefrom, vesicles or other structures formed from numerous of the cationic amphiphiles are not preferred by those skilled in the art in order to deliver low molecular weight biologically active materials" (Col. 33, lines 62-66) When the amphiphile is prepared as a thin-film evaporated from chloroform, "it may be advantageous to prepare the amphiphile-containing film to include one or more further ingredients that act to stabilize the final amphiphile/DNA composition," such as PEG-DMPE (Col. 53, lines 24-25 and 36-39).

MIHALKO ET AL. describe a method and system for inhalation administration of a drug in a suspension of liposomes, where the liposomes are formulated to produce a selected rate of drug-release from the liposomes (page 4, lines 16-20). The drug is released from the liposomes by efflux from the liposome into the respiratory tract (page 25, lines 9-13 and 20-22). The rate of efflux can be selectively controlled according to the lipid composition of the liposomes (page 4, lines 16-20). The drug release half-live may range from a half hour or less to six days or more (page 5, lines 6-12). The drug is subsequently taken up systemically from the site of deposition in the respiratory tract

(page 10, line 31 through page 11, line 2). The system further provides for a device to aerosolize the liposome suspension for inhalation (page 5, lines 13-17).

KLIBANOV ET AL. disclose coating liposome surfaces with a hydrophilic layer to create long-circulating liposomes (abstract). The liposomes are coated with the hydrophilic layer to avoid rapid uptake by the reticuloendothelial system (RES) (page 322, lines 11-12) for systemic delivery of the liposomes. The liposomes may further comprise an anti-tumor antibody attached (in one embodiment) to the distal ends of PEG chains on liposomes (page 331, lines 5-7). The purpose of the attached antibodies is to target the liposomes to a tumor site, for localized delivery of an entrapped drug at the site (page 330, lines 6-7). The only method of administration described is intravenous (page 330, lines 10-13).

GAO AND HUANG disclose a cationic cholesterol derivative as a nonviral transfection reagent (page 280, lines 11-12). Liposomes containing the cationic cholesterol derivative were found to be more efficient in transfection and less toxic to treated cells as compared to the lipofectin reagent (page 281, lines 1-3).

CHESTNUT ET AL. disclose compositions and methods for treating inflammation and other conditions using blocking P-selectin antibodies (abstract and Col. 2, lines 50-51). An anti-P-selectin immunoglobulin may be imbedded in an liposome to target the liposome to P-selectin molecules (Col. 21, lines 42-51).

DEFREES ET AL. relate to analogues of sialyl Le^x that inhibit cellular adhesion between a selectin and cells that express sialyl Le^x on their surfaces (Col. 1, lines 16-21). Liposomes with an entrapped chemotherapeutic agent can be targeted to a site of tissue injury by the selectin-SLe^x analogue (Col. 46, lines 46-49). The analogue is positioned on the surface of the liposome (Col. 47, lines 23-26). The liposome is fashioned such that a connector portion is incorporated into the membrane at the time

of forming the liposome membrane (Col. 47, lines 26-29). The connector portion has a lipophilic portion that is embedded and anchored in the membrane (Col. 47, lines 29-30). The liposomes may be administered parenterally or locally (Col. 49, lines 24-28).